Docket No.: 30610/39383

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(PATENT)

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of: Zankel *et al.*

Application No.: 10/600,862 Group Art Unit: to be assigned

Filed: June 20, 2003 Examiner: to be assigned

For: USE OF THE CHAPERONE RECEPTOR ASSOCIATED PROTEIN (RAP) FOR THE DELIVERY OF THERAPEUTIC COMPOUNDS ACROSS THE BLOOD BRAIN BARRIER

DECLARATION UNDER 37 C.F.R. § 1.132 OF DR. TODD ZANKEL

**MS AMENDMENTS**
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Dr. Todd Zankel, do hereby declare and state as follows:

1. I am a co-inventor of the invention claimed in the above-referenced application.

2. I am familiar with the contents of the above-identified U.S. patent application and I am providing this declaration to make available to the Examiner additional data relevant to the claims in the attached preliminary amendment.

3. The claims presented in the attached preliminary amendment are related to compositions that comprise Receptor Associated Protein (RAP) conjugated to enzymes involved in lysosomal storage disease and methods of using those compositions. Thus claims are directed to RAP fusion proteins and methods of using the same.

4. As discussed in the application at page 2, lines 5-15, lysosomal storage diseases (LSDs) are disorders that are characterized by the absence or reduced activity of specific enzymes within cellular lysosomes, resulting in the accumulation of undegraded "storage material" within the intracellular lysosome, swelling and malfunction of the

lysosomes, and ultimately cellular and tissue damage. Intravenous enzyme replacement therapy (ERT) is beneficial for LSDs (e.g. MPS I, MPS II). However, new compositions are required to achieve an efficient transfer of the delivered therapeutic enzyme into the lysosomes of the affected cells.

5. Exemplary LSDs that are known to require ERT include, for example, aspartylglucosaminuria, cholesterol ester storage disease, Wolman disease, cystinosis, Danon disease, Fabry disease, Farber lipogranulomatosis, Farber disease, fucosidosis, galactosialidosis types I/II, Gaucher disease types I/II/III, Gaucher disease, globoid cell leukodystrophy, Krabbe disease, glycogen storage disease II, Pompe disease, GM1-gangliosidosis types I/II/III, GM2-gangliosidosis type I, Tay Sachs disease, GM2-gangliosidosis type II, Sandhoff disease, GM2-gangliosidosis,α-mannosidosis types I/II, β-mannosidosis, metachromatic leukodystrophy, mucolipidosis type I, sialidosis types I/II mucolipidosis types II /III I-cell disease, mucolipidosis type IIIC pseudo-Hurler polydystrophy, mucopolysaccharidosis type I, mucopolysaccharidosis type II, Hunter syndrome, mucopolysaccharidosis type IIIA, Sanfilippo syndrome, mucopolysaccharidosis type IIIB, mucopolysaccharidosis type IIIC, mucopolysaccharidosis type IIID, mucopolysaccharidosis type IVA, Morquio syndrome, mucopolysaccharidosis type IVB Morquio syndrome, mucopolysaccharidosis type VI, mucopolysaccharidosis type VII, Sly syndrome, mucopolysaccharidosis type IX, multiple sulfatase deficiency, neuronal ceroid lipofuscinosis, CLN1 Batten disease, Niemann-Pick disease types A/B, Niemann-Pick disease, Niemann-Pick disease type C1, Niemann-Pick disease type C2, pycnodysostosis, Schindler disease types I/II, Schindler disease, and sialic acid storage disease. Such diseases may be treated by use of enzymes such as is selected from the group consisting of aspartylglucosaminidase, acid lipase, cysteine transporter, Lamp-2,α-galactosidase A, acid ceramidase, α-L-fucosidase, β-hexosaminidase A, GM2-activator deficiency, α-D-mannosidase, β-D-mannosidase, arylsulfatase A, saposin B, neuraminidase, _-N-acetylglucosaminidase phosphotransferase, phosphotransferase α-subunit, L-iduronidase, iduronate-2-sulfatase, heparan-N-sulfatase, α-N-acetylglucosaminidase, acetylCoA:N-acetyltransferase, N-acetylglucosamine 6-sulfatase, galactose 6-sulfatase, β-galactosidase , N-acetylgalactosamine 4-sulfatase, hyaluronoglucosaminidase, multiple sulfatases, palmitoyl

protein thioesterase, tripeptidyl peptidase I, acid sphingomyelinase, cholesterol trafficking, cathepsin K, α-galactosidase B, and sialic acid transporter.

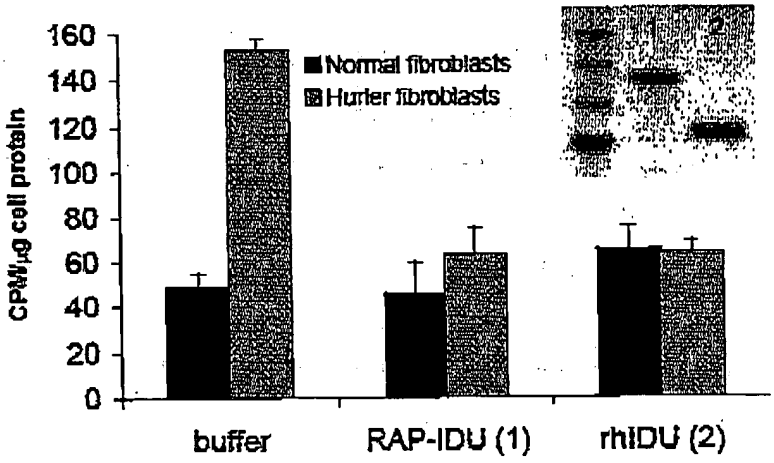
6. In order to achieve a more effective therapy of LSDs the invention describes the use of RAP fusion proteins. Such RAP fusions are prepared in order to effect delivery of the agent to which the RAP is fused. In the present claims, the agent to which the RAP is fused is an enzyme involved in lysosomal storage disease referred to herein as "lysosomal enzyme" or "LE." The RAP-LE fusions are designed to deliver active LE to the lysosome of a subject having lysosomal storage disease. The standard means of measuring lysosomal enzyme delivery and activity is to prevent accumulation of the enzyme's substrate in the lysosome by including enzyme in an affected cell's growth medium. In the present declaration, I provide data which demonstrates that an exemplary RAP-LE fusion, i.e., RAP fused to iduronidase (IDU) is able to be taken up by the lysosome and effect delivery of the IDU to the appropriate cellular site in order to effect a therapeutic outcome.

7. The data presented below were generated using either recombinant human IDU (rhIDU) alone or the RAP-IDU fusion. Both molecules prevent accumulation of stored substrate in the affected cells, demonstrating the competence of RAP to delivery active enzyme to the lysosome of the cell.

8. Human GM1391 Hurler fibroblasts were used in the studies to test the capabilities of RAP to delivery active LE to the lysosome. GM1391 cells were grown in DMEM containing 10% fetal bovine serum and 2 mM glutamine. Four days prior to the experiment, cells were seeded in 6-well plates at 250,000 cells per well. On the day of the experiment, cells were fed with sulfate-free medium (S-MEM, Irvine Scientific), 15% fetal bovine serum, 5 mM $CaCl_2$ for an hour and then the same medium supplemented to 4 μCi/mL with $^{35}S$-sodium sulfate and 5 nM of either RAP-IDU or rhIDU alone. Cells were incubated in this medium for 48 hours at 37°C in a humidified cell-culture incubator with 5% $CO_2$/95% air. Cell layers were rinsed three times with PBS before and after trypsinization. Pellets were lysed in 0.5N NaOH and neutralized with 1M HCl. Protein concentrations were determined by Bio-Rad Bradford assay in 96-well plates. Radioactivity in the lysates was counted in Beckman ReadyCaps™.

9. Experiments were performed to determine whether RAP-IDU could prevent the accumulation of glycosaminoglycan in patient fibroblasts. Hurler fibroblasts were grown in sulfate-free medium (S-MEM) in the presence of $^{35}$S-sulfate (Barton, R. W., and Neufeld, E. F. (1971) *J. Biol. Chem.* **246**, 7773-7779). RAP-IDU, rhIDU or buffer was included in the growth medium at a concentration of 5 nM. Cells were lysed and stored $^{35}$S-glycosaminoglycan was measured 48 hours later. Radioactivity in the cell lysates was normalized to total protein concentration in the same lysates. Total radioactivity per sample ranged from 4,000 to 20,000 cpm; total protein concentrations did not vary significantly between samples. Both RAP-IDU and rhIDU prevented $^{35}$S-GAG storage to the same extent, indicating that fusion is delivered to the lysosome and that fusion-derived IDU is competent to digest the natural substrate. The data are shown in the following Figure. The inset shows a 4-12% gradient Bis-Tris SDS-PAGE analysis of proteins used for experiment, stained with Coomassie Blue. RAP-IDU, lane 1; rhIDU, lane 2.

Figure.




10. It is my belief that the data presented in the above Figure show that a fusion of RAP-IDU will prevent the accumulation of glycosaminoglycan in Hurler fibroblasts, as does rhIDU.

11. These data with respect to RAP-IDU findings supplement the data provided in the specification, e.g., in Example IV which provides the measurement of specific uptake of RAP-conjugated human α-glucosidase (RAP-GAA) into enzyme-deficient patient fibroblasts. In Example IV and in Figure 13 of the specification, there is a teaching that RAP-GAA is taken up by GM244 fibroblast cells, a primary cell line isolated from a patient with glycogen storage disorder type II (Pompe's disease).

12. It is my opinion that the data presented in the specification as filed and corroborated by the present declaration show that RAP-LE fusions are useful in the treatment of LSDs. Mucopolysaccharidosis type I and Pompe's disease are exemplary LSDs from the class of LSDs listed in paragraph 5.

13. The data in the specification and the present declaration show that mucopolysaccharidosis type I and Pompe's disease may be treated using RAP-LE fusions in which the LE for mucopolysaccharidosis type I is IDU and for Pompe's disease is GAA. It is my belief that other RAP-LE fusions in which the LE is specific for a given LSD may readily be prepared and tested to produce additional corroborating results.

14. I further declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both (18 U.S.C. § 1001), and may jeopardize the validity of the application or any patent issuing thereon.

Date 12/9/04

Dr. Todd Zankel